# Possible Role of Cholecystokinin in the Development of Acute Pancreatitis in Rats

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Arch Jpn Chir 58(1), 3～17, Jan., 1989

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# Possible Role of Cholecystokinin in the Development of Acute Pancreatitis in Rats

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Received for Publication, Oct. 28, 1988.

The aim of this study was to elucidate whether cholecystokinin (CCK) had a role in the occurrence and/or in the development of experimental acute pancreatitis in rats, and furthermore to find the possibility for the treatment of acute pancreatitis with a CCK antagonist, proglumide. The administration of CCK-8 significantly increased serum levels of amylase, lipase and pancreatic wet weight. The administration of proglumide significantly reduced the blood levels of trypsin, pancreatic wet weight, water content and improved survival rate. These findings were supported by microscopic examination. The results of this study demonstrate that CCK has an important role in the development of acute pancreatitis and that proglumide might have prophylactic and therapeutic effects in acute pancreatitis.

## Introduction

Acute pancreatitis exhibits a broad clinical spectrum of symptoms and severity. It may present clinically as a mild case which resolves spontaneously or after conservative therapy but it may also be fatal by causing multiple organ failure (MOF)[34]. The etiology and the factors which may aggravate acute pancreatitis still remain to unknown[10] Treatment for acute pancreatitis has not been established. It has been suggested that the principle in traditional and conservative therapy of acute pancreatitis is not to stimulate but rather inhibit exocrine pancreatic secretion[22]. Stimulation of exocrine pancreatic secretion is believed to be an important factor in the pathogenesis of acute pancreatitis. Recent studies have also suggested that cholecystokinin (CCK), a humoral regulator of pancreatic enzyme secretion, plays an important role in the development of acute pancreatitis[32]. It is assumed then that the use of CCK receptor antagonist may prove to be beneficial in the treatment of acute experimental pancreatitis. We studied the role of CCK in the development of acute experimental pancreatitis in rats. The effect of proglumide, a CCK receptor antagonist, and the inhibitory action of proglumide

Key words: Experimental acute pancreatitis, Exocrine pancreatic secretion, Aggravating factor, Cholecystokinin, Proglumide.
索引用語：実験的急性膵炎，膵外分泌，重症化因子，コレシストキニン，プログルマイド.
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on exocrine pancreatic secretion in man were also studied. This study was performed to investigate the involvement of CCK in the pathogenesis of acute pancreatitis and to establish new principles of treatment for acute pancreatitis.

## Materials and Methods

### Animals

192 male Wistar rats weighing 150-200g were used in this study. During the experiment, 4 rats were kept in each cage in an environment with a constant temperature (22°C). The rats have free access to water and regular laboratory chow.

### Chemicals

Chemicals used in this study were as follows: proglumide; a kind gift from Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan), gabexate mesilate (FOY®); Ono pharmaceutical Co., Ltd. (Osaka, Japan), caerulein (Ceosunin®); Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), cholecystokinin octapeptide (CCK-8, KINEVAC®); Squibb Institute for Medical Research (Prinstone, NJ, USA), elemental diet (ED, Elental®); Morishita pharmaceutical Co., Ltd. (Osaka, Japan).

### Experimental models

**Model 1.** Caerulein induced acute pancreatitis

144 rats fasted for 18 hours were given 4 intraperitoneal (i.p.) injections of caerulein (20 $\mu$g/kg) at hourly intervals.

**Model 2.** Acute experimental pancreatitis caused by closed duodenal loop

48 rats fasted for 18 hours had a laparotomy via a midline incision under intraperitoneal sodium pentobarbital (40 mg/kg, Somnopentyl®, Pitmanmoore Co., Ltd. NJ, USA) anesthesia. A closed loop (3cm long) was created by ligating the duodenum at two points on both sides of the common bile duct. After the procedure, the abdominal wall was closed with a continuous monofilament string.

## Experimental Design

**Experiment 1:** Effects of CCK on caerulein induced acute pancreatitis (Model 1)

24 rats with caerulein induced pancreatitis (Model 1) were randomly divided into 2 groups, 12 in each group.

In group A, subcutaneous injection (s.c.) of CCK (2 $\mu$g/kg) was given 30 minutes after the last injection of caerulein. In group B, the control group, saline injections were given in the same manners in group A. 6 hours after the last injection of caerulein, all rats underwent laparotomy under ether anesthesia, and blood samples were collected from the inferior vena cava for measurement of serum amylase, lipase and trypsin. The whole pancreas was rapidly removed and weighed. Small pieces of the pancreas removed from the distal portion were weighed for the measurement of pancreatic water content. Pancreatic dried weight was measured after 72 hours of incubation at 80°C. Pancreatic water content was then calculated from these measurements. The rest of the pancreatic tissues were removed and fixed in a

10% buffered formalin solution for histological analysis.

**Experiment 2**: Effects of CCK and proglumide on acute pancreatitis caused by closed duodenal loop (Model 2)

48 rats were divided into 4 groups, 12 in each group. 10 minutes after the closed cuodenal loop operation, CCK-8 (2 $\mu$g/kg, s.c.) and proglumide (400 mg/kg, s.c.) were injected alone or together into 3 groups. To serve as control, one group received only saline injections using the same amount and method of administration as in the other group. Survival rate within the first 24 hours postoperatively was calculated in each group.

**Experiment 3**: Effects of proglumide or gabexate mesilate on caerulein induced acute pancreatitis (Model 1)

120 rats, divided into 10 groups, 12 in each group, were used in this study. Groups 1-5 were designated to be used for prophylactic treatment (prophylactic group) and groups 6-10 were for therapeutic treatment (therapeutic group). Group 1 served as the control for the prophylactic group. The rats were given intraperitoneal injections of saline 4 times at hourly intervals. Saline was also injected 30 minutes before the first and last injection of saline. Groups 2-5 received caerulein (20 $\mu$g/kg) 4 times at hourly intervals intraperitoneally. Group 2 served as the control for the caerulein induced pancreatitis in the prophylactic group. Saline was given 30 minutes before the first injection of caerulein. In group 3, rats were given 400 mg/kg proglumide 30 minutes before the first and last injection of caerulein. In group 4, 100 mg/kg gabexate mesilate was given 30 minutes before the first and last injection of caerulein. Group 5, proglumide and gabexate mesilate were given 30 minutes before the first and last injection of caerulein. In group 6, saline injections instead of caerulein were given 4 times at hourly intervals. Saline was also given twice, 3 hours apart, 30 minutes after the last of the hourly saline injections. Groups 7-10 were given caerulein 4 times at hourly intervals. In group 7, saline was given twice, 3 hours apart, 30 minutes after the last injection of caerulein. In group 8, 9 & 10, proglumide or gabexate mesilate was given twice, 3 hours apart, alone or in combination 30 minutes after the last injection of caerulein. Group 8 received only proglumide, group 9 only gabexate mesilate and group 10 both proglumide and gabexate mesilate.

**Experiment 4**: Effects of proglumide on pancreatic exocrine secretion in man

Radical pancreatoduodenectomy with Billroth II type of reconstruction was performed on 8 patients. In all cases, informed consents were obtained before the experiment. Chronic pancreatitis was not found in all cases. During the operation, a silicone tube was inserted into the pancreatic duct to drain pancreatic juice. A jejunal feeding tube was inserted into the jejunal lumen. The postoperative course in these 8 patients were uneventful. Experiments were done 14 days or more postoperatively. Saline (100 ml/h) was administered intrajejunally after a 14h fast. After the basal output of pancreatic juice secreted for 15 minutes was obtained, elemental diet (ED, 100 Cal/100 ml/h) or proglumide (400 mg/kg, 100ml/h) was administered alone or in combination intrajejunally. Pancreatic juice secreted was collected at 15 minutes intervals during the administration of the ED and/or proglumide. Pancreatic juice

volume, protein and bicarbonate were measured.

Biochemical determination

Blood samples were centrifuged at 3000 rpm for 15 minutes. Serum amylase concentration was determined by the G-5-CNP method using β-2-chrolo-4-nitrophenyl-maltopentaoside as substrate[43]. Serum lipase was determined by spectrophotometric method using 1-linoleoyl-2-palmitoyl glycerol as substrate(23). Serum trypsin concentration was determined using RIA gnost$^{R}$ Trypsin Kit (Hoechst Co. Ltd., West Germany). Protein concentration was determined using the Lowry method with bovine serum albumin as standard[27]. Bicarbonate concentration of the pancreatic juice was measured by the back-titrating method.

Histological analysis

For light microscopy, proximal pancreatic tissue specimens immersed in 10% buffered formalin were subsequently embedded in paraffin and sectioned into 4 μm slices. 100 sections at 40 μm intervals in each specimen were collected and stained with hematoxylin (H) and eosin (E). Histological alterations such as interstitial edema, vacuolization, inflammation, necrosis and hemorrhage were noted and analyzed [33]. The grading of the interstitial edema, inflammation and hemorrhage were graded to a scale which range from 0 as minimum to 4 as one with maximal changes. The histological grading of necrosis and vacuolization were according to the approximate percentage of cells involved[33]; 0=none, 1=<5%, 2=5-25%, 3=25-50%, 4=>50%

Statistical Analysis

Results were expressed as the mean±SEM. To analyze for statistically significant differences between means, the Student's t-test was used for the biochemical data and histological scores whereas the Kaplan-Meier test was used for the survival rates. Differences with a p value of $<0.05$ were considered significant.

## Results

**Experiment 1** (Table, 1 Fig. 1)

Rats injected with CCK-8, serum amylase, lipase, pancreatic wet weight and pancreatic water content increased significantly when compared to the rats which received only caerulein.

**Table 1.** All values represent mean±SEM.
Significant differences from caerulein group are indicated by *(=$p<0.05$).

| | Serum Amylase (SU/dl) | Lipase (IU/l) | Trypsin (ng/ml) | Pancreatic Wet Weight (% Body Weight) | Pancreatic Water Contents (%) |
|---|---|---|---|---|---|
| Caerulein | 26250 ± 1750 | 1255.0 ± 44.1 | 53.7 ± 1.4 | 0.72 ± 0.03 | 78 ± 2 |
| Caerulein +CCK$_8$ | 42052 ± 3554* | 1900.5 ± 104.5* | 58.4 ± 1.9 | 0.90 ± 0.02* | 83 ± 1* |

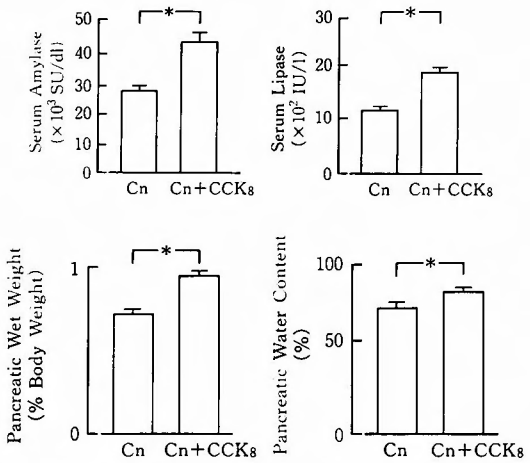



**Fig. 1** Effect of CCK-8 on serum amylase, lipase, pancreatic wet weight and pancreatic water content in rats with caerulein induced acute pancreatitis. *=p<0.05 compared to caerulein group.

**Experiment 2** (Fig. 2)

During the experimental period (24h), all rats of the control group, which were given saline or proglumide survived. Rats given CCK-8 displayed a significant lower survival ratio at the 12th and 19th hour compared to the rats which received CCK-8+proglumide. Survival ratio of the CCK-8+proglumide group was lower than the saline or proglumide groups.

**Experiment 3** (Table 2, 3, Fig 3, 4)

The prophylactic effects of proglumide (400 mg/kg s.c.) and gabexate mesilate (100 mg/kg s.c.) on caerulein induced pancreatitis are shown in Table 2, Fig 3. In the control group

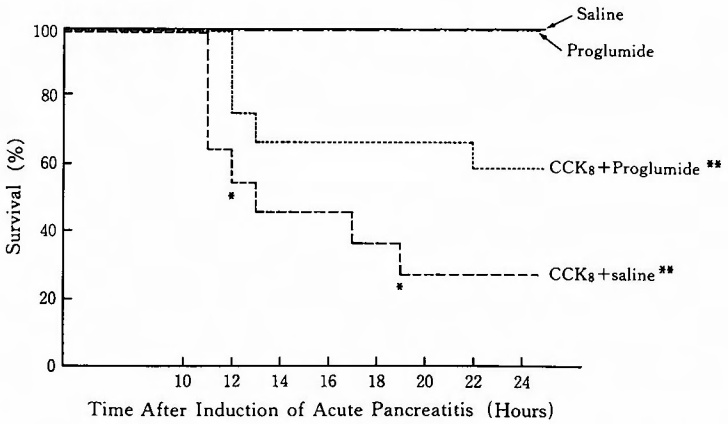
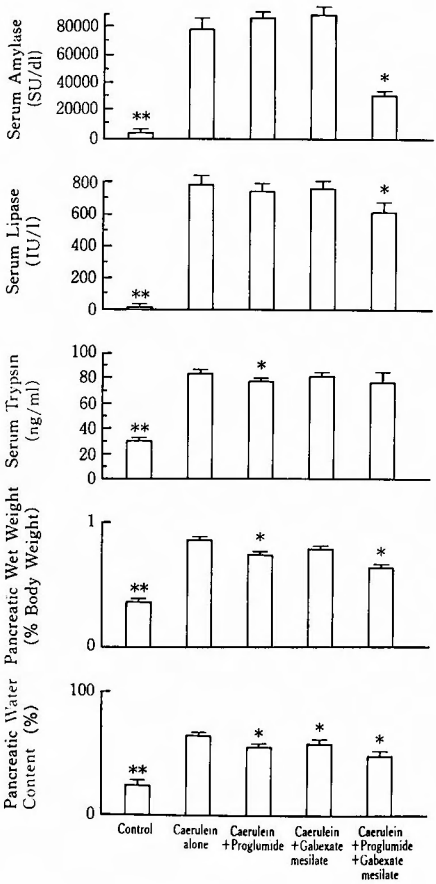



**Fig. 2** Cumulative survival ratio of the rats in 4 groups treated with saline, proglumide, CCK-8+proglumide and CCK-8+saline for 24hrs. after the closed duodenal loop operation (model 2).
n=12, *=p<0.05 compared to CCK+proglumide, **=p<0.05 compared to saline or proglumide.

**Table 2.**

| | Serum Amylase (SU/dl) | Lipase (IU/l) | Trypsin (ng/ml) | Pancreatic Wet Weight (% BW) | Pancreatic Water Contents (%) |
|---|---|---|---|---|---|
| Control | 3211 ± 192** | 9.9 ± 1.3** | 31.5 ± 1.2** | 0.37 ± 0.02** | 25 ± 3** |
| Caerulein alone | 78390 ± 9525 | 793.4 ± 86.9 | 84.2 ± 2.4 | 0.86 ± 0.03 | 66 ± 2 |
| Caerulein + Proglumide | 85820 ± 5200 | 744.8 ± 40.5 | 77.7 ± 1.8* | 0.73 ± 0.02* | 56 ± 2* |
| Caerulein + Gabexate mesilate | 88525 ± 8741 | 757.6 ± 48.7 | 81.6 ± 2.1 | 0.78 ± 0.02 | 58 ± 2* |
| Caerulein + Proglumide + Gabexate mesilate | 30193 ± 2928* | 609.5 ± 75.0* | 78.6 ± 4.6 | 0.63 ± 0.03* | 48 ± 3* |




Prophylactic effects of Proglumide (400mg/kg. s.c.) and Gabexate mesilate (100mg/kg s.c.) on caerulein induced acute pancreatitis.

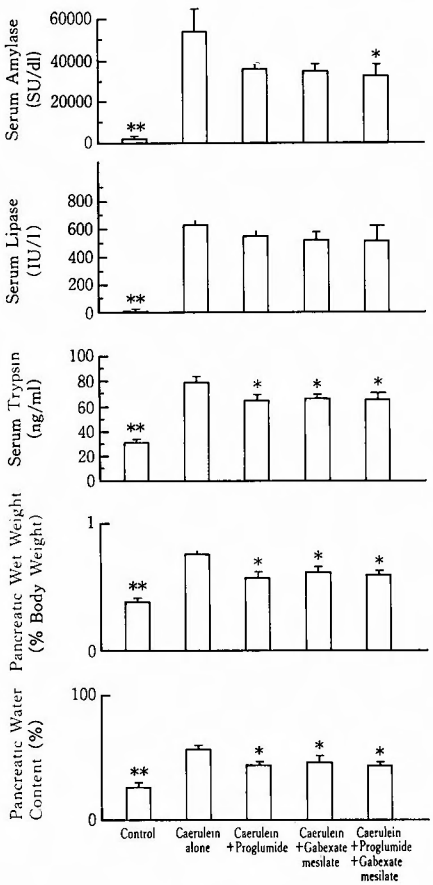
n=12, *=p<0.05 compared to caerulein only group, **=P<0.05 compared to all the other group.

**Fig. 3**

(group 1), rats injected with saline only instead of caerulein, serum amylase, lipase, trypsin, pancreatic wet weight and pancreatic water content were significantly lower than those of the caerulein treated groups with or without proglumide and gabexate mesilate. When compread

**Table 3.**

| | Serum Amylase (SU/dl) | Lipase (IU/l) | Trypsin (ng/ml) | Pancreatic Wet Weight (% BW) | Pancreatic Water Contents (%) |
|---|---|---|---|---|---|
| Control | 1863 ± 51** | 7.9 ± 0.5** | 32.0 ± 1.8** | 0.39 ± 0.02** | 28 ± 3** |
| Caerulein alone | 54126 ± 11699 | 683.9 ± 65.9 | 80.3 ± 4.1 | 0.76 ± 0.03 | 58 ± 2 |
| Caerulein + Proglumide | 36042 ± 2145 | 555.7 ± 42.0 | 65.9 ± 3.0* | 0.58 ± 0.03* | 45 ± 2* |
| Caerulein + Gabexate mesilate | 34906 ± 2563 | 539.0 ± 42.6 | 67.6 ± 2.2* | 0.62 ± 0.03* | 48 ± 3* |
| Caerulein + Proglumide + Gabexate mesilate | 32613 ± 4221* | 520.3 ± 117.0 | 63.8 ± 4.7* | 0.60 ± 0.02* | 45 ± 2* |




Therapeutic effects of Proglumide (400mg/kg s.c.) and Gabexate mesilate (100mg/kg s.c.) on caerulein induced acute pancreatitis.

n=12, *=p<0.05 compared to caerulein only group, **=p<0.05 compared to all the other groups.

**Fig. 4**

to the caerulein only group (group 2), the proglumide treated group (group 3) showed significantly lower values for serum trypsin, pancreatic wet weight and pancreatic water content. In the gabexate mesilate group (group 4), only the pancreatic water content was significantly

reduced. In the proglumide+gabexate mesilate group (group 5), serum amylase, lipase, pancreatic wet weight and pancreatic water content were significantly decreased.

The therapeutic effects of proglumide and gabexate mesilate on caerulein induced acute pancreatitis are shown in Table 3, Fig. 4. The serum amylase, trypsin, lipase, pancreatic wet weight and pancreatic water content of the control group (group 6) were significantly lower than those of the caerulein treated groups with or without proglumide and gabexate mesilate. Compared to caerulein only group (group 7), the proglumide treated group (group 8) and the gabexate mesilate group (group 9) showed significant reduction in serum trypsin, pancreatic wet weight and pancreatic water content. Likewise, serum amylase, trypsin, pancreatic wet weight and pancreatic water content were also significantly decreased in the proglumide+gabexate mesilate group (group 10).

**Table 4.** Prophylactic effects of Proglumide and Gabexate mesilate in caerulein induced acute pancreatitis on histological findings.
Significant differences are indicated by $*=p<0.05$ compared to caerulein alone group.

| | Interstitial edema | Vacuolization | Inflammation | Necrosis | Hemorrhage |
|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 |
| Caerulein alone | 2.00 ± 0.21 | 1.83 ± 0.17 | 1.67 ± 0.14 | 0 | 1.0 |
| Caerulein + Proglumide | 1.67 ± 0.26 | 1.33 ± 0.22 | 1.25 ± 0.31* | 0 | 0.83 ± 0.11 |
| Caerulein + Gabexate mesilate | 1.42 ± 0.15* | 1.55 ± 0.21 | 1.08 ± 0.15* | 0 | 1.17 ± 0.11 |
| Caerulein + Proglumide + Gabexate mesilate | 0.92 ± 0.15** | 1.17 ± 0.17** | 0.75 ± 0.13** | 0 | 0.42 ± 0.15** |

**Table 5.** Therapeutic effects of proglumide and gabexate mesilate in caerulein induced acute pancreatitis on histological findings.
Significant differences are indicated by $*=p<0.05$ compared to caelulein alone group.

| | Interstitial edema | Vacuolization | Inflammation | Necrosis | Hemorrhage |
|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 |
| Caerulein alone | 2.45 ± 0.21 | 2.45 ± 0.16 | 2.27 ± 0.14 | 0 | 1.09 ± 0.16 |
| Caerulein + Proglumide | 2.08 ± 0.19 | 1.92 ± 0.15* | 2.02 ± 0.12* | 0 | 0.92 ± 0.15 |
| Caerulein + Gabexate mesilate | 2.00 ± 0.21 | 1.67 ± 0.14* | 1.83 ± 0.11* | 0 | 0.83 ± 0.11 |
| Caerulein + Proglumide + Gabexate mesilate | 0.83 ± 0.11** | 1.25 ± 0.31** | 1.08 ± 0.12* | 0 | 0.50 ± 0.15** |

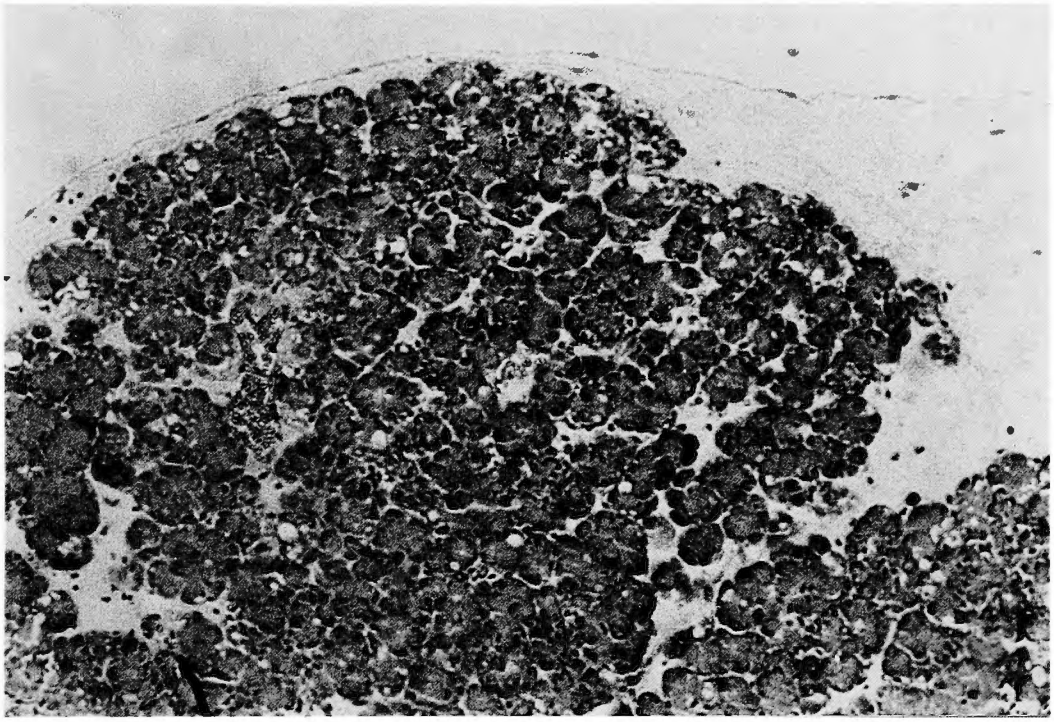


**Fig. 5** Microscopic picture of the rat pancreas after caerulein injection (20 $\mu$g/kg, i.p.). Marked interstitial edema, infiltration of leucocytes, cytoplasmic vacuoles and bleeding are seen. H-E staining. Original magnification $\times$200.

Histological findings (Table 4, 5, Fig. 5, 6)

Under light microscopy, interstitial edema, cytoplasmic vacuolization, inflammation and hemorrhage were seen in the pancreas of the caerulein treated rats. Interstitial edema was significantly reduced in the proglumide+gabexate mesilate group in both the prophylactic and therapeutic group. Cytoplasmic vacuolization was reduced in the proglumide+gabexate mesilate in the prophylactic group. The same was true for the proglumide, gabexate mesilate and proglumide+gabexate mesilate groups in the therapeutic treatment. Inflammation was reduced in the proglumide and/or gabexate mesilate groups in both the prophylactic and therapeutic treatment. Hemorrhage was reduced in the proglumide+gabexate mesilate group in both treatment.

**Experiment 4** (Fig. 7)

Intrajejunal (IJ) administration of ED significantly increased the volume of pancreatic juice and the amount of bicarbonate and protein secreted. IJ administration of proglumide (400 mg/kg) did not stimulate pancreatic secretion. IJ administration of ED+proglumide did not also stimulate pancreatic secretion.

## Discussion

The majority of patients with acute pancreatitis have a benign self-limiting disease. How-

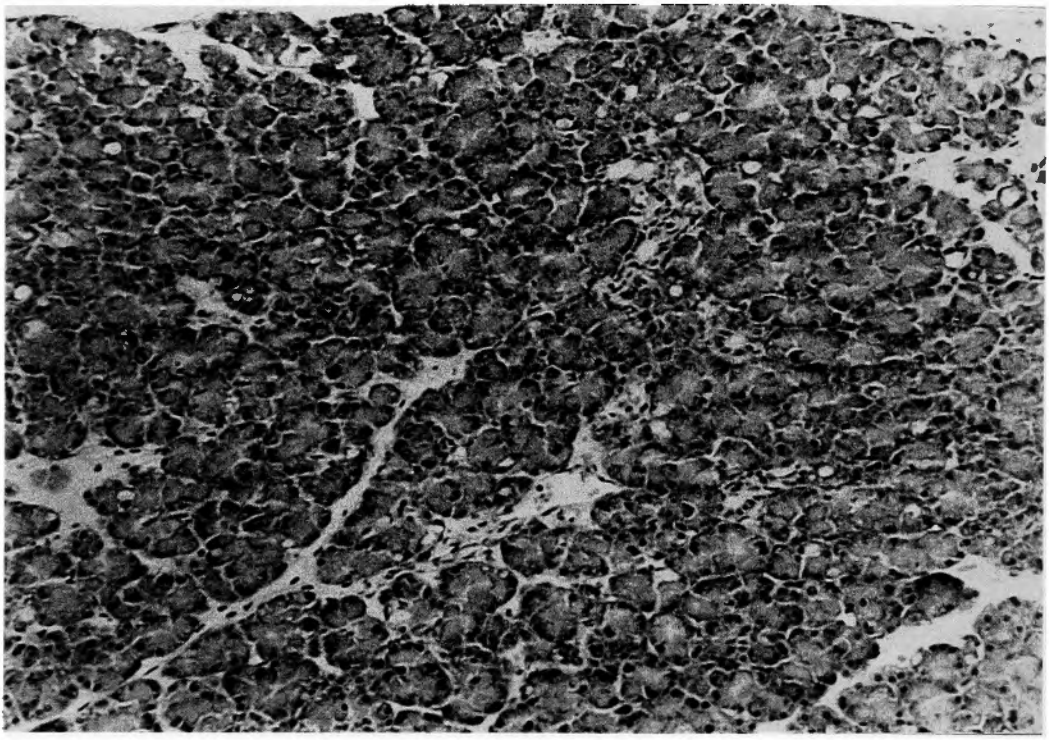


**Fig. 6** Microscopic picture of the rat pancreas after caerulein injection (20 μg/kg, i.p.) with therapeutic treatment (group 7) with proglumide (400mg/kg, s.c.). Slight interstitial edema and some cytoplasmic vacuoles are seen, but leukocytes infiltration or bleeding did not occur. H-E staining. Original magnification ×200.

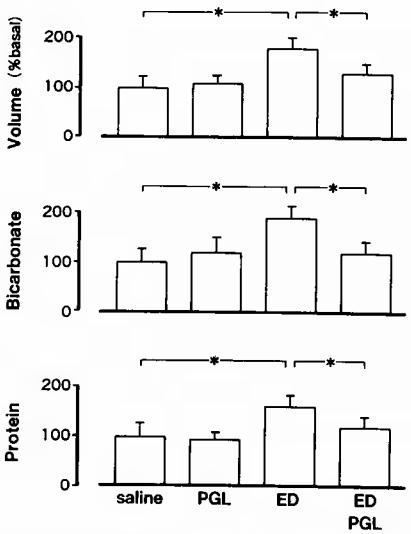



**Fig. 7** Pancreatic juice volume, bicarbonate and protein outputs in response to intrajejunal ED and/or proglumide. n=8, *=p<0.05. ED; elemental diet PGL; proglumide.

ever, a few cases lead to severe hemorrhagic necrotizing pancreatitis and eventually to MOF. It is generally accepted that activation of pancreatic enzymes within the pancreas leading to autodigestion is essential for the pathogenesis of acute pancreatitis [10]. There have been many factors, known or unknown, postulated to produce and aggravate pancreatitis. Some of the etiologic factors and mechanisms in pancreatitis include biliary tract disease, alcoholism, trauma, surgery, tumors, metabolic derangements, drugs and others[7,34]. It was reported that the severity of acute pancreatitis is unrelated to the etiology[44]. Therefore, it has been suggested that some factors may play an aggravating role after the establishment of pancreatitis. Whereas, what factors precipitates acute pancreatitis is still unknown.

CCK is a gastrointestinal hormone with a potent stimulatory effect on exocrine pancreatic secretion[11,40] and protein synthesis[24], and has a most important role as a humoral regulator of exocrine pancreatic secretion and enzyme secretion. Therefore, it was assumed that CCK might play an important role in the development of acute pancreatitis. Measurement of plasma CCK in rats by radioimmunoassay has been difficult, so little was done in the investigation of CCK in acute pancreatitis. Recently, CCK receptor antagonist[20] have been shown to elucidate the action and the role of CCK without the actual measurement of plasma CCK. Using a specific CCK-antagonist, proglumide[19], we investigated the role of CCK in the pathogenesis and the development of acute experimental pancreatitis. Proglumide is a derivative of glutamic acid and has been used for 10 years in the treatment of peptic ulcer diseases[18]. The mechanism of the effect of proglumide has not been elucidated, but studies on pancreatic acini demonstrated that proglumide works as a specific receptor antagonist of CCK-related peptides[19,20]. It has been shown to have the same effects on exocrine pancreatic secretion in vivo[15,30,38] and gallbladder contraction in vitro[4] and in vivo[17].

Proglumide is considered to be useful in defining the roles of CCK in the modulation of several pathophysiological functions[5,9]. The dose of proglumide (400 mg/kg s.c.) in this study was chosen since it was effective in inhibiting pancreatic stimulatory actions of CCK in man[38]. Today, the principle of treatment in acute pancreatitis is the inhibition of exocrine pancreatic secretion by fasting and draining of digestive juice[22], and of the activation of digestive enzymes using protease inhibitors such as gabexate mesilate[16,21,37]. However, substances such as atropine, glucagon and somatostatin, which inhibit pancreatic enzyme secretion, do not have beneficial effects in acute pancreatitis[6,12,45]. NIEDERAU et al. reported that blockade of CCK receptors and early inhibition of protease activity may be beneficial in severe acute pancreatitis in mice[32]. It has also been suggested that CCK antagonists might act by protecting plasma membrane and stimulus-secretion coupling in intracellular events[32]. In this study, 2 experimental models of acute pancreatitis were used. One is mild edematous pancreatitis induced by a large dose of caerulein[25], and the other is hemorrhagic necrotizing pancreatitis caused by closed duodenal loop[31]. EVANDER et al. reported that during acute experimental pancreatitis, caerulein administration increased the mortality rate and the incidence of ascites and the amylase activity in ascites in rats[13]. Caerulein is a synthetic decapeptide that was originally extracted from the skin of the Australian amphibian Hyla caerulea[3]. Caerulein shares seven

of its eight C-terminal amino acids with the C-terminal octapeptide of CCK and has an effect on exocrine pancreatic secretion similar to that of CCK[29]. It has been reported that intravenous, intraperitoneal or subcutaneous administration of large doses of caerulein causes acute interstitial pancreatitis in rats and mice[25,42]. This model has subsequently been used as a model to study the mechanism of pancreatitis as well as to study effects of potentionally protective agents[1,2,46]. LAMPEL et al[25]. reported that intravenous infusions of caerulein (5 μg/kg/h) induced interstitial pancreatitis in rats. NIEDERAU et al. reported that intraperitoneal injections of caerulein (50 μg/kg, 7 times hourly) induced necrotizing pancreatitis in mice[33]. TANI et al[42]. reported that subcutaneous injections of caerulein (5, 10, 20, 50 μg/kg, 4 times hourly) induced edematous pancreatitis at the dose of 20 μg/kg and 50 μg/kg without significant differences between the two doses. In this study, rats received 4 injections of caerulein (20 μg/kg) intraperitoneally at hourly intervals and acute interstitial pancreatitis developed in all cases.. Acute pancreatitis model caused by closed duodenal loop is considered to be due to reflux of biliary juice and duodenal content including active enzymes into the pancreatic duct[31]. Clinically, this type of acute pancreatitis is observed in afferent loop syndrome after gastrectomy with the reconstruction of gastrojejunostomy (Billroth Ⅱ) in which the duodenum was not anastomosed[28]. It has been suggested that this model for experimental pancreatitis has several biochemical, morphological as well as clinical similarities to the human necrotizing pancreatitis[26,39].

In both experimental models of acute pancreatitis, the administration of CCK-8 worsened acute pancreatitis in rats, when compared to controls (Fig. 1, 2). The dose chosen (injection of 2 μg/kg s.c.) did not, by itself, cause any biochemical or histological evidence of acute pancreatitis in normal conditions[32]. Thus, CCK, even at physiological concentrations, appears to play an important contributory role in the development of acute pancreatitis. Our data support the statement that CCK plays an important role in the development of acute pancreatitis as previously reported[32]. Thus, it was considered that the blockade of CCK receptors might have some effects in the prevention of the occurrence and development of acute pancreatitis.

Proglumide had beneficial effects on serum enzymes, histologic alterations in caerulein induced acute pancreatitis, and on survival in closed duodenal loop pancreatitis (Table 2, 3, Fig. 2, 3, 4). It was suggested that blockade of CCK receptors with proglumide inhibits the occurrence and the development of acute pancreatitis. To clarify the role of CCK in the pathogenesis or in the development of acute pancreatitis, expriment 3 was designed. In the prophylactic group, proglumide was administered before the induction of caerulein. This was designed to investigate the role of CCK in the pathogenesis of acute pancreatitis. To investigate the role of CCK as an aggravating factor in acute pancreatitis, proglumide was administered after induction of experimental pancreatitis. In addition, gabexate mesilate, a protease inhibitor, which is clinically used in acute pancreatitis in man, was administered alone or in combination with proglumide, to evaluate the prophylactic and therapeutic effect in acute pancreatitis. Gabexate mesilate is a synthetic protease inhibitor and has been shown to inhibit the enzymatic activity of pancreatic enzymes such as trypsin, kallikrein, plasmin, thrombin, C1-elastase and phospholipase $A_2$[14,16,21]. It has been shown to cause a reduction

of mortality in rats in which acute pancreatitis was induced by closed duodenal loop[41] In this study, supramaximal dose of gabexate mesilate (100 mg/kg s.c.) was used to evaluate the beneficial effect in acute experimental pancreatitis in rats. Prophylactic or therapeutic administration of proglumide alone in caerulein induced acute pancreatitis improved not only the concentration of serum trypsin, pancreatic wet weight and pancreatic water content, but also histologic alterations in acute pancreatitis. Simultaneous administration of proglumide and gabexate mesilate reduced further the levels of serum amylase, lipase and also showed improvement in the histologic alterations in acute pancreatitis (Table 4, 5). It was suggested that the blockade of CCK receptors by using proglumide inhibits the trigger action of CCK in the pathogenesis and the development of acute pancreatitis. The dose of proglumide (400 mg/kg) used in the study was the same dose used in an in vivo study in man and it was found that it did not increase pancreatic enzyme secretion stimulated by endogenously released CCK (Fig. 7).

However, this amount of proglumide is actually too much to use in clinical practice. Therefore, more potent CCK antagonists such as CR-1505[36] or L364, 718[8], which has been recently developed, need to be looked into further for possible use in the therapy of acute pancreatitis. It may also be necessary to combine these substances and potent protease inhibitors in the treatment of severe pancreatitis. From the data gathered, the use of CCK antagonists in the treatment of acute pancreatitis should be looked into further. Recent studies have also suggested that superoxide dismutase[35] and phospholipase $A_2$[14] are aggravating factors in acute pancreatitis. Hence, further investigations of the relationship between CCK and these substances are also necessary.

In conclusion;

1) CCK may be an important factor in the development of acute pancreatitis.
2) CCK antagonist has prophylactic and therapeutic effects on acute experimental pancreatitis.
3) CCK antagonist might be an effective therapeutic drug for acute pancreatitis.

## Acknowledgements

We thank Ms. Mayumi Okamoto, Ms. Akiko Suzuki and Ms. Minori Kamuro for their excellent technical assistance and Prof. Yoshio Okada and Prof. Atsumi Mori for their suggestions and advices.

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## 和文抄録

# ラット急性膵炎におけるコレシストキニンの役割について

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本研究はコレシストキニン (CCK) が急性膵炎の発症，あるいはその進展に関与しているのか，そして更には CCK の拮抗物質が，急性膵炎の治療薬になりうるのかを調べる目的で行った．実験は Wistar 系ラットを用い，急性膵炎に果たす CCK，および CCK の特異的受容体拮抗剤であるプログルマイドの効果を検討した．本実験では二つの膵炎モデル，すなわちセルレイン膵炎，および十二指腸結紮による急性膵炎モデルを用いた．セルレイン膵炎では，CCK-8の投与を行うことにより血中アミラーゼ，リパーゼ，膵湿重量が有意に増加した．同じ膵炎モデルにおいて，プログルマイドの投与により血中トリプシン，膵湿重量そして膵含有水分量は有意に減少し，また十二指腸結紮による急性膵炎モデルでも生存率を有意に改善した．これらの結果は，実験に用いたラット膵の組織学的検討でも確かめられた．以上より，CCKは実験的急性膵炎の発症および進展に，重要な役割を持っていることが明かとなった．そして CCK 拮抗剤（プログルマイド）は急性膵炎の予防と治療に有用であることが示唆された．